

57 Quantification of CFTR IVS8(T) splice variants in CAVD patients

L. Sousa¹, A. Grangeia¹, F. Carvalho¹, M. Sousa^{1,2}, A. Barros^{1,3}. ¹Faculty of Medicine of Porto, Genetics, Porto, Portugal; ²Lab Cell Biology, ICBAS, U. Porto, Porto, Portugal; ³Center Reprod Genetics A. Barros, Porto, Portugal

Apart of CF, CFTR gene mutations have also been associated with CFTR-related disorders, such as congenital absence of the vas deferens (CAVD). The most common CAVD mutation is the IVS8(T)5 variant in CFTR gene intron 8 that alters the efficiency with which the splice acceptor is used. The aim of the present study was to analyze and compare the levels of CFTR mRNA transcripts lacking exon 9 obtained from testicular and nasal biopsies of CAVD patients.

A total of 23 testicular biopsies were analyzed: 12 from CAVD and 10 from patients with conserved spermatogenesis (controls). In 4 CAVD patients, nasal biopsies were also analyzed. The relative amounts of accurately spliced transcripts (+9) and transcripts lacking exon 9 (−9) were evaluated by real-time PCR, using a multiplex based assay (TaqMan).

We detected in all samples the presence of the 2 CFTR transcripts, +9 and −9. However, patients carrying IVS8(T)5 variant produced higher proportions of transcripts −9 than patients carrying the IVS8(T)7 or IVS8(T)9. Additionally, it was observed that the proportion of transcripts −9 was higher in testicular biopsies than in the nasal epithelium of the same patient.

As previously reported, we confirmed that the degree of CFTR exon 9 skipping was inversely correlated with the IVS8(T)n polymorphic tract. Moreover, the level of CFTR mRNA transcripts lacking exon 9 was increased in testicular biopsies when compared with nasal biopsies from the same individual. Thus, differential efficiency between different tissues expressing CFTR may explain the reproductive tract abnormalities, such as CAVD observed in our patients, and absence of pathologic changes in other CF-associated organs.

59 Abnormal TRAF4 and TMEM16a expression during tracheal development in CFTR-deficient mice

L. Guillot¹, M. Ruffin¹, L. Riffault¹, E. Bonvin¹, P. Le Rouzic¹, O. Tabary¹, M. Bonora¹. ¹INSERM UMR-S 938 – UPMC Paris 06, CdR St-Antoine – Equipe Capeau/Clement, Paris, France

We have previously demonstrated that adult and newborn cystic fibrosis (CF) mice exhibit tracheal cartilage ring abnormalities (Bonvin *et al.*, 2008). In the present study, we explored two different molecular mechanisms possibly involved in these congenital malformations based on (1) the influence of secondary genetic factors during airway development, (2) a reduction in Cl[−] secretion into the fetal lung liquid. We investigated the expression of the TRAF4 gene which is required for normal development of the trachea, and the TMEM16a gene, a dominant calcium-activated Cl[−] channel in mouse airways, which is expressed in tracheal epithelium and smooth muscle. The expression level of these two genes was examined by qPCR in total embryos and *ex-vivo* tracheas at embryonic stage E15 in *Cftr* knockout and F508del-CFTR mice and their respective controls.

Our data show a lower expression of TRAF4 mRNA in embryos and tracheas at E15 in the two CF mice compared to their controls. We also found that the expression of TMEM16a mRNA is similar in total embryos of CF mice as compared to controls, but is significantly larger in tracheas alone. These results support the hypothesis that TRAF4 should play a role in the occurrence of tracheal abnormalities and suggest that TMEM16a may provide a parallel route for Cl[−] secretion for trachea that lack CFTR although this effect is insufficient to avoid cartilage defects. Our present findings prompt us to investigate further the role of these two genes in congenital malformations of the trachea within the context of CFTR protein dysfunction.

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Reference(s)

Bonvin *et al.*, J. Physiol 586, 2008.

58 Molecular analysis of CFTR gene in 27 fetuses with hyperechogenic bowel

M.J. Sá¹, A. Fortuna¹, M. Rocha¹, J. Silva¹, M. Barbosa¹, O. Pinho¹, T. Delgado¹, P. Pacheco², G. Soares¹. ¹Medical Genetics Unit, Centro Genética Médica Jacinto Magalhães – INSA, Porto, Portugal; ²Genetics Department, Molecular Biology Unit – INSA, Lisboa, Portugal

Background: Hyperechogenic bowel (HB) is defined as a fetal bowel with equivalent brightness to the iliac crests, occurring in 0.1–1.8% of pregnancies. It is an echographic marker of fetuses at increased risk for cystic fibrosis (CF), chromosomopathies and fetal infection. Association with other anomalies may enable recognition of a syndrome. It can be a normal variant (65%) or a consequence of intraamniotic bleeding.

Objective: To perform a retrospective analysis (2005–2009) of CFTR gene analysis in fetuses with HB, referred to our center.

Methods: Clinical cases were selected from clinical and laboratory databases, using the terms “hyperechogenic bowel” and “echographic marker”. Clinical data included family history, second trimester ultrasound examination, CFTR gene screening, fetal karyotype, maternal and/or fetal serologies (toxoplasmosis, rubella and cytomegalovirus), and pregnancy outcome. Genetic counselling was performed in all cases.

Results: Family history was irrelevant in all 27 cases. Isolated HB occurred in 24 cases (88.9%), and association to pelvic dilatation in 3 cases. CFTR gene analysis identified 1 carrier couple of the mutation F508del, and their homozygotic fetus (3.7%). Two other carriers of mutations G542X and G576A were identified. 1 fetus had a 47,XY,+21 karyotype (3.7%) and concurrently a cytomegalovirus infection (3.7%). No other infections were observed. Follow-up in 7 cases (25.9%) was known: 5 healthy newborns and 2 terminations of pregnancy.

Discussion: HB is a significant echographic marker that should be evaluated in the context of genetic counselling since it can lead to the identification of affected fetuses and carrier status for CF.

60* Identification of TRPC6 as a novel potential target to activate CaCC in human CF airway epithelial cells

J. Bertrand¹, F. Antigny¹, L. Dannhoffer¹, C. Jayle², F. Becq¹, C. Vandebrout¹, C. Norez¹. ¹Institut de Physiologie et Biologie Cellulaires, Université de Poitiers, Poitiers, France; ²Service de Chirurgie Cardiothoracique, CHU Poitiers, Poitiers, France

One possible target for drug discovery aiming at treating Cystic Fibrosis is to correct the ionic imbalance through stimulation of alternative ionic pathway that may compensate the deficient CFTR dependent Cl[−] secretion. In previous studies, we identified guanabenz as an activator of Ca²⁺ dependent Cl[−] channel (CaCC) via a Ca²⁺ influx in human CF nasal (CF15) and tracheal (CF-KM4) epithelial cell lines and in freshly isolated CF and non CF human ciliated epithelial cells. Here, we explored the molecular mechanism of Ca²⁺ signaling induced by guanabenz and hypothesis that a member of the TRP channel family could be the target of guanabenz.

The profile of expression of TRPC and TRPV isoforms revealed the presence of TRPC1, C6, V1, V2 and V3 in both CF-KM4 and CF15 cell lines. We demonstrated that the guanabenz induced-response was inhibited by SKF, a TRPC family inhibitor but not by Ruthenium Red, a TRPV family inhibitor. Moreover, we demonstrated that a Small interfering RNA (SiRNA) TRPC6 transfection reduces by 45–50% i) the expression of TRPC6 ii) the guanabenz-dependent Ca²⁺ influx and iii) the CaCC-dependent activity stimulated by guanabenz.

In conclusion, we demonstrated that TRPC6 could be considered as one of the molecular target of guanabenz. However, it remains to study the possible involvement of TRPC1 in guanabenz induced response. These results highlighted TRPC6 as a novel potential target to activate CaCC in CF human airway epithelial cells.

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